

ON SOME TREMATODE PARASITES OF MARSUPIALS  
AND OF A MONOTREME.

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Plates lxxv.-lxxvi.)

The small group of Trematodes, here under discussion, comprises two new species of *Harmostomum* from marsupials, and two species of *Mehlisia*, one from a marsupial, also described as new, and one from the platypus, *Ornithorhynchus anatinus* Shaw, described by me in these Proceedings in 1901, under the designation *Distomum ornithorhynchi*(7). The two species of *Harmostomum* are closely related to one another; but, what is more interesting, are also closely related to *H. opisthotriasis* Lutz, parasitic in the South American marsupial, *Didelphys aurita*(13); so nearly, in fact, as I shall show in detail later on, that they must be looked upon as derived from a common ancestor; and, in this way, they supply some circumstantial evidence of the phylogenetic relationship of the Australian and the South American marsupials.

HARMOSTOMUM DASYURI, sp.n.

(Plate lxxv., Figs. 1, 5-8.)

Parasitic in the intestine of *Dasyurus viverrinus*, found in the vicinity of Sydney, N.S.W.

*Diagnosis*.—Small, flattened worms, rounded in front, pointed behind, about 6 mm. long, by 1.25 mm. broad. Integument armed with spines, except on the posterior half of the dorsal surface. Suckers very large, the ventral almost as wide as the body; *ratio of the oral to the ventral sucker*, 2:3. Small prepharynx, well developed pharynx, and short œsophagus present. Intestinal limbs with a forwardly directed loop, thence running back to the extreme posterior end of the body. Excretory vesicle very small in its unpaired part, dividing into two long branches, *giving off numerous*

*vessels*. Gonads oval, *comparatively large*, in the posterior end of the body, the anterior testis with its *long axis transversely, posterior with its long axis longitudinally disposed*; ovary lying between the two testes, with its long axis transversely placed. Genital pore near the anterior edge of the anterior testis. Copulatory organs well developed, but *cirrus-sac moderate*, and not surrounding the vesicula seminalis. Laurer's canal present, but no receptaculum seminis. Uterus in ascending and descending coils, not reaching anteriorly beyond the ventral sucker, nor posteriorly beyond the front of the anterior testis. Yolk-glands lying at the sides of the body, between the ventral sucker and the anterior testis; just reaching inwards beyond the intestinal limbs.

Eggs dark-shelled, oval, flattened on one side, small, 0.0254 × 0.0165 mm.

Type-specimen in the Australian Museum, No. W. 352.

I received eight specimens of this worm, all obtained from one host, from the Bureau of Microbiology, Sydney; of these, two were sectioned, and the others mounted whole. The host, which was sent in to the Bureau by Mr. Thomas Steel, came from Hunter's Hill, a suburb of Sydney. These moderately small worms, 6.33 mm. in average length, and 1.24 mm. in breadth, have a somewhat flattened, elliptical cross-section, remain fairly broad, and are rounded off in front; but, behind the gonads, taper to a point posteriorly. The cuticle is fairly thick, and the ventral surface and the sides are thickly beset with small pointed spines, which gradually become finer posteriorly, but are present right up to the posterior end. On the dorsal surface, however, they practically vanish at the level of the posterior edge of the ventral sucker. Both the suckers are large. The oral sucker is subterminal, with its opening directed ventrally; the average diameter is 0.67 mm. The ventral sucker, lying in the first body-third, is almost as broad as the worm's body, its average diameter being 1.001 mm., so that the ratio of the diameter of the oral sucker to that of the ventral, is 2:3. The pharynx, which is preceded by a short præpharynx, is a large muscular organ, practically circular in ventral view, and having a diameter just one-half that of the oral

sucker. There is a short œsophagus, 0.08 mm. long. The intestinal limbs run at first anteriorly, as far as the oral sucker, and then run backwards, in a straight or slightly wavy course, to the extreme posterior end of the body. Looss(8) says, of *H. æquans* Lss., that these forwardly running loops are caused by the contracted state of the body; but I cannot agree with this, in regard to the similar loops found in the two species of *Harmostomum* described in this paper, but regard them as very characteristic and peculiar features of their alimentary canal. My specimens show no signs of unusual contraction, and there seems to be no reason why the slight, but inevitable contraction brought about by ordinary methods of fixing and preserving, should cause this peculiar feature in these worms and their relations, but in no others.

The excretory system is peculiar, and corresponds, in its main features, to that of *H. leptostomum* Olss., described by Looss (9, p. 168). The median, undivided part of the excretory vesicle, opening at the posterior end of the body, is very short, only 0.16 mm. long; but it divides into two branches that run to the level of the oral sucker, and, bending round, course back once more to the posterior end, giving off numerous capillary branches, that end in flame-cells, along their whole length; but the flame-cells are more numerous at the anterior end than elsewhere. A special feature of these vessels, which Looss, in the case of *H. leptostomum* Olss., regards as branches of the vesicle itself rather than ordinary collecting tubes, is that, for part of their course, they possess little patches of cilia projecting into their lumen.

The genital pore is situated on the ventral surface, in the middle line, just in front of the anterior testis. The testes, which are large oval bodies lying in the posterior third of the body, seem to be characteristically placed, the anterior with its long axis transversely or slightly obliquely across the body, while the posterior has its long axis lying directly longitudinally in regard to the body of the worm. They are equal in size, and measure 0.57 mm.  $\times$  0.46 mm. The ovary, which lies between them, and is sometimes slightly overlapped by them, is also oval in shape, with its long axis transversely placed. The vasa deferentia come off from the middle of

the anterior surface of each testis respectively; that from the posterior skirts round the lateral aspect of the ovary and anterior testis (Fig. 5), and joins its fellow just after it leaves the anterior testis. The tube, formed by their union, shortly enters the vesicula seminalis, which is a coiled tubular body, of comparatively considerable length, not enclosed in the cirrus-sac, but lying free in the parenchyma. The cirrus-sac, a moderately developed muscular sac, surrounding the cirrus with its ejaculatory duct, does not extend inwards beyond the junction of the ejaculatory duct with the vesicula seminalis. The prostate cells lie free in the parenchyma. The cirrus-sac and vagina, which has thick muscular walls, are surrounded by modified parenchyma cells.

The ovary ( $0.39 \times 0.31$  mm.) is smaller than the testes, and lies either median or slightly lateral. Like the testes, it is a smooth oval body. The oviduct leaves it in the middle of its ventral surface (Fig. 6), and runs to its posterior edge; here the ootype is situated, and just in front of this, Laurer's canal takes its origin, and curves round to the dorsal surface, where it opens on the exterior (Fig. 6). There is no receptaculum seminis. The "shell-glands" round the ootype are not well developed. The yolk-reservoir lies close to the ootype. From the ootype, the female duct runs forwards in a slightly coiled course to the level of the genital opening, and this part of it, which is stuffed full of sperms, is the receptaculum seminis uterinum; it then runs, in a series of transversity placed loops in the ventral surface of the body, as far forwards as the middle of the ventral sucker, returning in the dorsal part of the body by a series of complex folds to end at the genital opening. The loops of the uterus laterally lie strictly within the inner boundary of the intestinal limbs.

The yolk-glands consist of numerous, quite small, oval follicles (average size  $0.038 \times 0.023$  mm.), which lie in the lateral aspect of the body outside the intestinal limbs; in front, they just reach the level of the posterior edge of the ventral sucker, while posteriorly, they do not pass beyond the level of the anterior testes. Their ducts form two conspicuous lines that at first gradually, then more quickly, converge on the yolk-reservoir.

The small dark-shelled eggs ( $0.0254 \times 0.0165$  mm.) have the characteristic elliptical shape, flattened on one side, and are very numerous.

HARMOSTOMUM SIMILE, n.sp.

Parasitic in the intestine of the bandicoot, *Perameles obesula*, found in the vicinity of Sydney, N.S.W. (Fig. 2).

*Diagnosis.* — Small worms, oval in cross-section, rounded in front, pointed behind, about 3 mm. long, by 0.5 mm. broad. Integument spiny on the ventral and lateral surfaces, but smooth on the dorsal surface, *except at the extreme anterior end*. Suckers comparatively large, *ratio of the oral to the ventral sucker, 3:4*. Alimentary and excretory systems as in *H. dasyuri*. Gonads oval: anterior testis *smaller than posterior*, fairly globular in form. Genital pore near the anterior edge of the anterior testis. Cirrus and vagina well developed and muscular, but *cirrus-sac very poorly developed*, Laurer's canal present, but no receptaculum seminis. Uterus and yolk-glands as in *H. dasyuri*. Eggs very small,  $0.0219 \times 0.0129$  mm.

Type-specimen in the Australian Museum, No. W. 353.

I have received four specimens of this worm, one from the Bureau of Microbiology, Sydney, and three from my friend, Dr. Harvey Johnston, of the University of Queensland. Two were sectioned, and the others mounted whole.

This is obviously closely related to the foregoing species, pretty closely corresponding in its anatomical structure, but differing from it in its much smaller size, in the relative sizes of the suckers, in being less spiny, in its more weakly developed cirrus-sac, in the anterior testis being smaller than the posterior, and subglobular, and in the size of the eggs, which are smaller in *H. simile*. In the latter, too, the testes are nearer the posterior end, so that there is practically no tail. All four worms were about the same size, the average length being 2.98 mm., breadth 0.52 mm. The diameter of the oral sucker averages 0.273 mm., that of the ventral 0.372 mm. The pharynx, with a diameter of 0.155 mm., is comparatively larger than in *H. dasyuri*. The anterior testis measures 0.218 mm. in average diameter, the posterior  $0.272 \times 0.239$  mm., the ovary  $0.227 \times 0.187$  mm.

These two species are obviously very closely related to *H. opisthotrias*, Lutz, parasitic in a South American marsupial, apparently more closely related to it than to any other species of *Harmostomum*. They differ from it chiefly, as far as one can judge from the descriptions given by Lutz (13), and later by Braun (2) (though Braun probably had to do with another species), in the very different relations in size of the suckers, in the different relative sizes of the testes, in the size of the eggs, and several other very minor points. But these three (or four) species from marsupials agree so closely in so many characteristic features, such as the whole structure of the alimentary tract, and the whole configuration of the genital system, that they should be looked upon as having been derived from common ancestors. These species from marsupials agree together, and differ from the other known species of *Harmostomum*, as *H. leptostomum* Olss., *H. æquans* Lss., parasitic in mammals; and *H. fuscatum* R., *H. marsupium* Brn., *H. centrodes* Brn., *H. mordens* Brn., *H. caudale* R., parasitic in birds, in the fact that neither the coils of the uterus, nor the yolk-glands extend anteriorly beyond the ventral sucker.

A second species of worm from *Dasyurus viverrinus*, and that parasitic in the dudodennm of the platypus, I have found it necessary to refer to a new genus.

#### MEHLISIA,\* gen. nov.

Fasciolid trematodes of moderate size, elongated and narrow, leaf-like, thick; widest just behind the ventral sucker, with a constriction in front of this (Fig. 3). Integument spiny, cuticle thick. Suckers large, near together. Præpharynx, pharynx, no œsophagus, intestinal limbs extending to near posterior end. Excretory vesicle with main stem reaching testis, and dividing into four longitudinal branches that form an anastomosing network in anterior end of body, opening into a sinus surrounding oral sucker; a similar sinus round ventral sucker; ciliated vessels and supplementary vessels present. Genital pore near midline, in front of ventral sucker. Copulatory organs present. Testes large and

\* Named from the gland of Mehlis, which is of very large size.

elongated, in middle of body; ovary in front of testes to one side of the middle line. Laurer's canal present; no receptaculum seminis. "Shell-gland" very large. Uterus short. Yolk-glands extensive, laterally placed in front of the testes; behind the posterior testes spreading under the whole surface of the body. Eggs very large. In the intestine of lower mammals.

MEHLISIA ACUMINATA, sp.n.

(Figures 3, 9, 10.)

Parasitic in the intestine of the marsupial "cat," *Dasyurus viverrinus*.

*Diagnosis*.—Size moderate; form elongate, narrow leaf-shaped, tapering to a sharp point behind. Integument spiny; cuticle thick. Suckers very large, near together; ratio of oral to ventral, 1:2. Præpharynx, pharynx, no œsophagus, simple intestinal limbs, reaching the posterior end. Excretory system of vessels and sinuses as in the genus. Copulatory organs present. Genital opening just in front of ventral sucker. Testes one behind the other in the middle of the body; ovary in front of the testes on one side of the middle line, much smaller than the testes. Laurer's canal present, but no receptaculum seminis. "Shell-gland" very large. Uterus short, restricted to the middle field between the ovary and ventral sucker and intestinal limbs. Yolk-glands extending from the posterior edge of the ventral sucker to the posterior end, at first laterally placed, behind the posterior testis spreading over the whole surface of the body. Eggs very large,  $0.134 \times 0.079$  mm., few in number.

Type-specimen in the Australian Museum, No. W.355.

I have received about 40 specimens of this trematode, some from Dr. J. P. Hill, of the University of London, and formerly of the University of Sydney, some from Dr. Harvey Johnston, of the University of Queensland, and some from the Bureau of Microbiology, Sydney, all taken from the intestine of the marsupial "cat," *Dasyurus viverrinus*, collected in various parts of New South Wales. In shape, these worms are elongated and narrow, leaf-like, flattened dorsoventrally, but the flatness varies consider-

ably, and in preserved specimens the shape in transverse sections in almost invariably somewhat flattened oval. They are widest just behind the ventral sucker, rounded in front, but gradually tapering to a long point behind; there is a slight constriction near the ventral sucker, just in front of the widest part of the body. The average size is 11.2 mm., long, by 2.34 mm. wide.

The cuticle is thick and tough, beset with numerous sharp-pointed spines, closely set in the anterior region of the body, but gradually becoming sparse and scattered towards the posterior extremity. In the body-wall, the longitudinal muscles are arranged in columns separated from one another by connective tissue cells (Fig. 9).

The suckers are very large; the oral spherical, with its aperture ventrally placed near the anterior end; the ventral, placed close to the oral, is also spherical, nearly as wide as the body, and deeply implanted in it. The average diameters are, oral 0.66 mm., ventral 1.32 mm.; ratio of oral to ventral, 1:2. The relative size is very constant, being practically the same in every specimen measured.

The pharynx, much smaller than the oral sucker, joins it through a præpharynx of length rather less than its own. There is practically no œsophagus, and the intestinal limbs run out to the sides of the body, and then proceed, in a pretty straight course, to end a little distance in front of the posterior extremity. They are quite simple in form, showing no signs of lateral cæca.

The excretory system of vessels is very richly developed, and exhibits some very marked peculiarities. The main stem of the vesicle, which opens by a small pore at the extreme posterior end, is a long wide tube reaching up to the level of the posterior testis, where it divides into four longitudinal branches that run forwards to the anterior end of the body. Both from the main stem and its four branches, a series of short lateral branches proceed towards the surface, and break up into a number of branches that lie in the parenchyma among the yolk-cells, many of them reaching the inner aspect of the cuticle. In this respect, as in others to be pointed out below, they resemble the condition found in *Mesaulus grandis* R., (Braun, 5). The four

longitudinal vessels, given off from the stem of the vesicle, end in front in an anastomosing network of vessels, which is connected, by a fairly large opening (Fig. 10) on each side, with a large sinus surrounding the oral sucker. A similar sinus also surrounds the ventral sucker. Similar sinuses surrounding the suckers have been found by Braun (2, 5) in *Mesaulus grandis* R., and in *Echinostoma incrassatum*, Dies., but their connection with the excretory system was not observed (5, p. 27). All these vessels, including the network and the sinuses, are lined in the same way by a nucleated syncytium. Given off from them are smaller intracellular tubes that end in flame-cells. The latter are more numerous at the anterior end of the body.

Opening into the sinus that surrounds the oral sucker, I find a pair of tubes of quite different character. The walls are thicker, and elongated patches of cilia (Fig. 9) project into the lumen. The intervals between these groups of cilia are small, so that the tube is ciliated in the greater part of its length. These two tubes run backwards, one on each side, to a level some distance behind the testes, where each passes into a tube lined by columnar nucleated cells, but bearing no cilia. These latter tubes, which I have marked "supplementary tubes" in Fig. 9, run forwards, parallel to their ciliated companions, and backwards to the posterior end of the body where they bend round, and are continued forwards again for some distance. At about the level where they disappear, a second forwardly running branch is given off from the parent supplementary tube, and this second branch reaches a level somewhat in front of the junction of the supplementary tube with the ciliated tube. The supplementary tube and its branches gradually become smaller and thinner-walled, and finally end in a system of intercellular spaces lying in the parenchyma. The function of all these tubes and sinuses is, no doubt, respiratory as well as excretory, and the fluid in their cavities is kept in circulation by the ciliated tubes.

The genital pore is situated on the ventral surface, near the middle line, just in front of the ventral sucker. The gonads lie close together, occupying about the middle third of the body-

length. The testes, which lie in the middle line, one behind the other, are very large, 1.69 mm. long by 0.59 mm. broad, roughly crescentic or S-shaped, with an irregular, indented outline. The ovary is rounded or oval in shape, with a smooth outline, much smaller than the testes, close in front of the anterior, and a little to one side (the right) of the middle line. The ducts of the testes run forwards, laterally placed, one on each side of the uterus, then dorsal to the ventral sucker; they join at the base of the cirrus-sac, and immediately enter the S-shaped vesicula seminalis. The cirrus-sac is a muscular-walled, pear-shaped body of considerable size; the prostate cells lie in its parenchyma surrounding the proximal part of the ejaculatory duct, into which their fine ducts open. The oviduct leaves the ovary on its dorsal aspect, and soon gives off Laurer's canal, which, after a short curved course, opens on the dorsal surface near the middle line. Just distal to its junction with Laurer's canal, the oviduct expands into the ootype, and here the duct of the yolk-reservoir opens into it. The next portion of the female duct is frequently filled with sperms, and is the receptaculum seminis uterinum. The "shell-gland" or gland of Mehlis, is a large mass of elongated cells with fine ducts opening into the ootype. Not only the ootype, but also the yolk-reservoir and Laurer's canal lie embedded in this mass of gland-cells. There is no receptaculum seminis. The uterus, which is comparatively very short, lies in the middle field of the body between the ovary and the ventral sucker, being disposed in several transversely placed coils, which do not reach laterally the intestinal limbs. The final part of its course, lying dorsal to the ventral sucker, is fairly straight. The vagina or metraterm has its muscular walls only moderately developed.

The follicles of the yolk-glands are small ( $0.069 \times 0.056$  mm.), and exceedingly numerous. They form compact masses, lying, at first, at the sides of the body, but, at the level of the posterior testis, they begin to spread over to the middle, and behind the testes they fill up the whole field, forming a complete layer under the surface of the body (Fig. 3). Anteriorly, they do not extend beyond the posterior edge of the ventral sucker. There is a longitudinal yolk-duct on each side, lying near the outer side of the

intestinal limbs, receiving numerous tributaries from the follicles as it passes (Fig. 9). The anterior and posterior parts of these ducts meet at the level of the anterior edge of the anterior testis, and, from the point of junction on each side, a transverse duct proceeds to meet its fellow of the other side, in the mass of the shell-gland.

The elliptical eggs are light-yellow in colour, very large ( $0.134 \times 0.079$  mm.), and comparatively few in number.

#### MEHLISIA ORNITHORHYNCHI mihi.

(Figs. 4 and 11.)

Parasitic in the duodenum of the platypus, *Ornithorhynchus anatinus*.

*Diagnosis*.—Elongated worms, tapering somewhat, but rounded off at each end. Integument spiny, spines larger than in *M. acuminata*. Suckers large, near together; *ratio of oral to ventral*, 2:3. Præpharynx, pharynx, no œsophagus, simple intestinal limbs extending to the posterior end. Excretory system of vessels and sinuses as in the genus. Copulatory organs present. Testes elongated, lobulated, *obliquely placed* in the middle line, one behind the other. Ovary, uterus, shell-gland, Laurer's canal and receptaculum seminis as in *M. acuminata*. Vitelline glands as in *M. acuminata* in regard to their extent, but consisting of *larger follicles*. Eggs  $0.13 \times 0.069$  mm., few in number.

Type-specimen in the Australian Museum, No. W. 354.

I have already published an account of this species, in the Proceedings of this Society (7), where a figure of the worm is given. In that figure, the structures marked *d.s.g.*, should be the Laurer's canal. Fig. 4 in this paper is a corrected presentment of the female organs. The general anatomy, and especially the excretory system, are very similar to the descriptions given for *M. acuminata*. It differs from that species principally in the shape of the body, in its smaller size, in the larger size of its spines, in the ratio of the suckers, in the oblique position and more elongated form of the testes, and in the larger and less numerous follicles of the yolk-glands.

*Relationship.*—These two species seem to me to be related, on the one hand to the Fasciolinæ, and, on the other, to the Echinostominæ and Psilostominæ. They should be looked upon, I think, as members of a sub-family Mehlisiinæ, with, for the present, the characters of the genus, intermediate in position between those groups. They differ from the Fasciolinæ in their form and size, in the simpler form of the gonads and intestinal limbs, for the most part, and especially in the character of their excretory apparatus; while they differ from the Psilostominæ in the absence of a receptaculum seminis, the very large size of the gland of Mehlis, the larger and more complex form of the testes, in the form of the excretory system in some respects, and in the more extended disposition of the yolk-glands. They find their nearest relatives, perhaps, in such forms as *Cotylotretus rugosus* Odhn., (14), and *Mesaulus grandis* R.(5). In determining the phylogenetic relationships of Trematodes, it should be borne in mind that they were parasitic in the early ancestors of the vertebrates, and, owing to the conditions under which their lives are passed, have been much less subjected to evolutionary changes in form than their hosts, so that we should rather expect to find the nearest relatives of trematode parasites of primitive mammals amongst such groups as the Echinostominæ and Psilostominæ parasitic in birds and reptiles.

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#### EXPLANATION OF THE PLATES.

Figures 1-3 are drawings of whole-mounts, made with the help of the camera lucida. Figure 4 is a diagram compiled from a series of transverse sections. These drawings were made by Mr. F. W. Atkins, of the Sydney Technical College. Figures 5-11 are untouched up photographs of sections.

#### Reference letters.

*C.s.*, cirrus-sac—*C.v.*, ciliated vessel of the excretory system—*Ej.d.*, ejaculatory duct—*E.*, excretory vessel—*E.s<sub>1</sub>.*, excretory sinus round the oral sucker—*E.s<sub>2</sub>.*, excretory sinus round the ventral sucker—*G.p.*, genital pore—*G.c.*, genital chamber—*Int.*, intestinal limbs—*L.c.*, Laurer's canal—*L.y.d.*, longitudinal yolk-duct—*M.l.*, longitudinal muscle—*M.c.*, circular muscle—*O.*, ovary—*O.c.s.*, opening of ciliated vessel into oral sucker sinus—*O.e.v.*, opening of excretory vessels into the sinus—*O.d.*, Oviduct—*Oot.*, ootype—*P.*, penis—*Ph.*, pharynx—*P.Ph.*, præpharynx—*Pr.*, prostate—*R.s.*, receptaculum seminis—*R.s.u.*, receptaculum seminis uterinum—*S.g.*, “shell-gland”—*S.v.*, supplementary vessel—*T.*, testis—*T.y.d.*, transverse yolk-duct—*Ut.*, uterus—*Ut.a.*, ascending loop of uterus—*Ut.d.*, descending loop of uterus—*Vag.*, vagina—*V.d.*, vas deferens—*V.s.*, vesicula seminalis—*Y.d.*, yolk-duct—*Y.g.*, yolk-glands—*Y.r.*, yolk-reservoir.

Fig. 1.—*Harmostomum dasyuri*, from the intestine of the marsupial “cat,” *Dasyurus viverrinus*.

Fig. 2.—*Harmostomum simile*, from the intestine of *Perameles obesula*.

Fig. 3.—*Mehlisia acuminata*, from the intestine of *Dasyurus viverrinus*.

Fig. 4.—Diagram of the female organ of *Mehlisia ornithorhynchi*.

Fig. 5.—Horizontal longitudinal section through the posterior end of *H. dasyuri*; (× 45).

Fig.6.—Transverse section of *H. dasyuri* through ovary, oviduct, and Laurer's canal;( $\times 110$ ).

Fig.7.—T.S., *H. dasyuri* ( $\times 180$ ).

Fig.8.—T.S., *H. dasyuri*; $(\times 90)$ .

Fig.9.—T.S., *Mehlisia acuminata*, showing ciliated vessel and supplementary vessel of the excretory system; $(\times 160)$ .

Fig.10.—T.S., *M. acuminata*, showing the sinus round the sucker, as well as a number of vessels of the network, together with their opening into the sinus; $(\times 40)$ .

Fig.11.—T.S., *M. ornithorhynchi*, showing the arrangement of the muscle layers, the very large shell-gland, etc.; $(\times 55)$ .